Finding time

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Finding time
A daily clock in yeast

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Circadian (circa 24 h) clocks confer a temporal structure on biological processes.[1] Although they have been largely defined based on the surprising quality of a free-running rhythm with a frequency of about a day, in nature these timers serve to segregate various processes to different—presumably optimal—times of the 24 h day. Circadian clocks are cell-based, meaning that although the clock in an animal can be discerned through the timing of behavior, circadian rhythms are also apparent in each cell of the pacemaker in the brain, the suprachiasmatic nucleus.[2] They are also ticking in skin cells, organ cells and cells that have been in tissue culture for decades.[3]

Beyond the animal kingdom, clocks have turned up in plants (*Arabidopsis thaliana*) and in fungi (*Neurospora crassa*).[4] Even prokaryotes have a clock model organism, namely photosynthetic cyanobacteria. It seems that wherever we look, we can expect to find a clock, even in 'lower organisms. However, there remain many model systems for which no obvious free-running circadian rhythm has been demonstrated. This could reflect the absence of a circadian clock, but experiments from the Johnson lab show that one cyanobacterial circadian clock out-competes another within ten generations.[5] Thus, it seems that most organisms will readily capture spontaneous mutations that confer improvements in temporal order.

Another explanation for failure to find free-running circadian rhythms is a bit trivial: the conditions that are permissive for circadian rhythm have not yet been identified. For many organisms that are robustly rhythmic, there are probably more ways to make them non-rhythmic than rhythmic! Animals and fungi in constant light and the model system *Neurospora* in a bona fide wild type background appear non-rhythmic. It is difficult to predict what the appropriate, permissive, constant conditions are, thus an alternative regime could be a useful. Given that the clock is designed to confer timing in the context of entrainment (synchronization to 24 h signals—zeitgebers—from the external environment), this circadian property could be used to demonstrate a biological timer.

Remarkably, circadian entrainment mirrors the entrainment of physical oscillators with each other as described by Huygens in the 17th century.[6] As oscillators couple to each other (like when the biological clock is coupled to the light/dark cycle) they assume a characteristic phase relationship that depends on the properties of both oscillator systems.[7] In a long cycle, a circadian oscillator will synchronize earlier than in a short one. In a cycle with a weak entraining stimulus, the circadian phase will also shift within the cycle. A non-circadian or driven synchronization will fail to show this systematic entrainment, rather recurring at the same phase independent of the structure of the zeitgeber.[8]

The circadian field would benefit tremendously from having the budding yeast, *S. cerevisiae*, in our toolkit but many of us have failed over the years in our efforts to discover the clock in this cell. Thus, we developed a system for following entrainment for weeks or months at a time using chemostat cultures.[9] We used temperature cycles as an entraining zeitgeber and modified standard methods (e.g., leaving pH unregulated, to be used as a readout of the state of the cell cultures). Under thus optimized conditions, we could clearly see evidence of circadian—non-driven—entrainment. When the cultures were released from entrained conditions to a free-run, they would typically show one or two oscillations before damping out to constant levels. This is either an indication that the conditions for a free-run are still sub-optimal or that yeast really does not have a self-sustained rhythm as so many other biological clocks do. Regardless of the capacity to free-run, the entrainment experiments show that there is an endogenous mechanism in yeast that is capable of moving processes to specific times of day depending on conditions. In other words, yeast has a clock.

Concerning the clock mechanism in yeast, they have no homologs of known 'clock genes' but there are several proteins that are predicted to have PAS domains, a protein sequence that is found in virtually all eukaryotic clock gene networks.[10] These may eventually be shown to play a role in the yeast clock. Another approach to elucidating the clock mechanism involves building on the extensive knowledge of cell biology: the clock-regulated pH oscillations are likely derived from nitrogen metabolism. When key permeases were assayed for expression level, they support this theoretical framework (see **Fig. 1**): under temperature entrainment and also in the free run, the ammonium permease, MEP2, and the general amino acid permease, GAP1, are expressed several hours ahead of the acidification of the media. The regulation of the permeases could be a downstream, clock-controlled output of the circadian clock mechanism, or maybe in finding time in yeast, we'll find some truly novel regulatory tricks.

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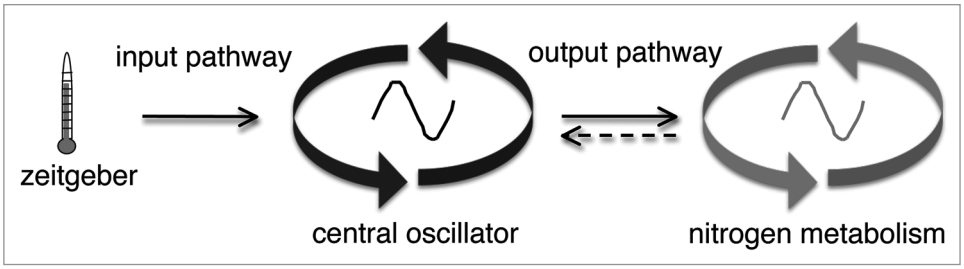

Figure 1. Temperature cycles and entrainment of the circadian clock in yeast. Circa 24 h temperature cycles result in a systematic synchronization pattern as dictated by a central oscillator. The manifestation of clock function is oscillations in pH of the media, thus implicating nitrogen metabolism, which could simply be downstream of the clock (right). Alternatively, it could feed back onto the clock system.

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